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Comparative Studies of the Antigenic Polypeptide Species VP4, VP6, and VP7 of Three Strains of Bovine Rotavirus

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Three bovine rotavirus strains belonging to two distinct serotype groups, serotype 6 (NCDV and B641) and B223, distinct from the other six mammalian rotavirus serotypes but not yet assigned to a serotype group, were compared with each other and with canine rotavirus (K9, serotype 3) by studying the properties of their cognate polypeptide species VP4, VP6, and VP7. The three viruses showed distinct differences in the polyacrylamide gel electrophoretic migration rates of protein species VP4 and VP7, with minor differences in VP6. Differences were also observed among the migration patterns of genome segments 4, 6, and the 7-8-9 triplet, which encode VP4, VP6, and VP7, respectively. Monoclonal antibodies (MAbs) to B223, which were directed against VP4 or VP7, showed homologous specificity for neutralization and immunofluorescence (IF), although one MAb reactive with VP4 also reacted by IF and by immunoprecipitation (IP) with all four viruses and weakly neutralized B641 and K9. This MAb may react with the epitope responsible for the B223-induced one-way neutralizing and protection response of calves against B641 observed in earlier studies. MAbs reactive with VP6 by IP showed enzyme-linked immunosorbent assay and IF reactivity with all three bovine viruses and the canine virus. The two serotype 6 viruses could be distinguished by the two B641 MAbs, B641-N2b reacting by neutralization and IF with both viruses and B641-N1 reacting with B641 and the serotype 3 canine rotavirus but not with NCDV. One nonneutralizing B641 MAb reacted by IP and IF with VP7 of all four rotaviruses examined, and one B223 MAb neutralized B223 and, to low titer, B641 and K9, although reacting by IP and IF with all four viruses. Three MAb-resistant mutants were selected by passage of B223 in the presence of one of three selected B223 MAbs at concentrations which only neutralized approximately 90% of the infectious virions. The resulting mutants were 100% resistant to neutralization with their respective MAb but remained neutralizable by the same selection of MAbs as the parent B223 virus.

The three bovine rotavirus isolates NCDV, B641, and B223 can be subtyped according to their serotypic and electrophoretic properties. NCDV and B641 have been placed in the same serotype group, US bovine serotype 1 (26), or rotavirus serotype 6 (15), although they exhibit minor antigenic differences. In contrast, B223 was placed in a distinct serotype, US bovine rotavirus serotype 2 (26). B223 is not neutralized by antisera used to serotype the other six mammalian serotypes of rotavirus (H. F. Clark, Wistar Institute, Philadelphia, Pa., personal communication; G. N. Woode, unpublished data) but has not been assigned to a rotavirus serotype group. All three viruses can be distinguished by their electropherotypes (26).

Studies of active immunity have demonstrated that a complex antigenic relationship exists among the bovine viruses. NCDV-vaccinated calves produced B641-neutralizing antibodies in their serum and intestines (coproantibodies), but they were not protected against challenge with B641. In contrast, calves vaccinated with B223, although a different serotype, did develop low-titer heterologous neutralizing antibodies to B641 and were protected against challenge with B641. This was a one-way reaction, as calves vaccinated with B641 neither developed neutralizing antibodies to B223 nor were protected against challenge with B223 virus (26, 27). Bridger and Oldham (3) also have demonstrated a lack of correlation between serotype and active immunity. However, most studies have shown that active immunity to rotavirus infections in a number of species is associated with the serotype specificities of rotaviruses, in these cases suggesting a specific role for the outer capsid antigens of the viruses (reviewed in references 14 and 26).

VP4 and VP7 are outer capsid proteins encoded by genome segments 4 and 7, 8, or 9, respectively, depending on the strain of rotavirus (16), and antibodies to either protein neutralize rotavirus (17). Thus, these antigens are the determinants of serotype specificity, passively acquired immunity, and passive protection against challenge (18, 19, 21). The results of homologous and heterologous challenge experiments with NCDV, B641, and B223 show one-way heterologous cross-protection among the three bovine rotaviruses and suggest that the antigens stimulating the production of neutralizing antibodies are not always involved in the development of active immunity. A recent report indicates that cytolytic T lymphocytes from immunized animals recognize both homologous and heterologous rotavirus-infected target cells in vitro (20). This suggests a possible role for cytolytic T lymphocytes in heterologous protection from rotavirus infection, although T-cell-deficient mice show normal recovery from experimental rotavirus infection (8). Similarly, cytolytic T lymphocytes recognizing nonstructural antigens of heterotypic influenza A viruses have been reported, and these cytolytic T lymphocytes appear to be involved in heterologous protection (2).

To assist our investigations into the determinants of serotype specificity and active immunity in bovine rotaviruses, we developed monoclonal antibodies (MAbs) against the viral antigens VP4, VP6, and VP7 of B641 and B223, with the expectation that common and strain-specific epitopes might be identified among the three bovine rotavirus strains. In

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addition, the proteins of NCDV, B641, and B223 were compared by polyacrylamide gel electrophoresis (PAGE). Canine rotavirus (10), which is a serotype 3 rotavirus sharing the common rotavirus antigen (VP6) with the bovine rotaviruses (11), was included as a control.

MATERIALS AND METHODS

Rotavirus strains. The origins of the bovine rotaviruses have been described previously (26). The canine rotavirus (K9) was isolated (10) and cloned by plaque selection, and its antigenic relationships were determined as a serotype 3 rotavirus (11, 15).

Reassortant viruses were constructed by crossing B223 and the 4F variant of SA11 (serotype 3) isolated by Pereira et al. (22) in MA104 cells. The yield of virus was plated at high dilution, well-isolated plaques were picked, and titers were amplified by passage on MA104 cells. The parental origin of genome segments in the progeny clones was determined by electropherotype analysis on high-resolution gels as described previously (12, 27). Progeny clones with desired electropherotypes were sequentially plaque purified three times, and the electropherotype was confirmed before use. The B223/SA11-4F reassortants used in these studies reassorted SA11-4F genome segment 4 (VP4), 6 (VP6), or 9 (VP7) in various combinations on a background of segments derived from B223.

Genome RNA electropherotyping. The methods used for genome RNA electropherotyping have been described previously (12, 27). Briefly, virus obtained from infected cultures was ultrapelleted through 40% sucrose at 100,000 × *g*, the RNA was extracted with phenol and chloroform, and genome segments were analyzed by sodium dodecyl sulfate (SDS)-PAGE in 7.5% gels.

Rotaviral polypeptides. The method of Ericson et al. (9) was used for the intracellular radiolabeling of rotavirus polypeptides with [^{35}S]methionine and their extraction. The polypeptides were analyzed by SDS-PAGE (5% stacking and 10% separating polyacrylamide gels under constant current).

MAb production. BALB/c mice were immunized with either B223 or B641 which had been semipurified and prepared with Freund complete adjuvant, followed 3 weeks later with virus and Freund incomplete adjuvant. Immunizations continued until the enzyme-linked immunosorbent assay (ELISA) titer in serum reached 5×10^5 to 1×10^6 log 10 and the neutralization test (NT) titer was greater than 20,000. At 10 and 3 days prior to fusion, the mice were inoculated with nonadjuvanted virus intravenously or intraperitoneally. The spleen cells (10^8 to 10^9 log 10) were isolated and mixed with SP2/0 myeloma cells and mouse peritoneal macrophages in the respective proportions of 300:100:1. After centrifugation at 100 × *g*, 0.5 ml of 40% polyethylene glycol was layered onto the cell pellet, gently stirred, and incubated at 37°C for 1 min. Then 10 ml of selective hybridoma medium (hypoxanthine-aminopterin-thymidine) was added dropwise, followed by 10 ml quickly, and then the suspension made up to 200 ml with hypoxanthine-aminopterin-thymidine medium. The cells were dispensed in 10 96-well microdilution plates.

Cell-free fluids from the hybridomas which originated from preferably one colony were screened by NT, immunofluorescence (IF), and ELISA at dilutions of 1:2, 1:5, and 1:10. NT and ELISA were performed with homologous antigens. For IF, the homologous reaction was first tested, and if it was positive, the hybridoma was retested with heterologous (canine) rotavirus antigens.

The hybridoma cultures producing rotaviral MAbs were cloned three times. All MAbs used in this study were produced in tissue culture.

Antibody assays. The following assays were used to characterize the MAbs and the B223 postinfection gnotobiotic calf serum GC47 (26).

(i) NT. The NT has been described previously (26). Approximately 500 immunofluorescent focus-forming units (IFFU) of virus were incubated with antibody dilutions for 1 h at 37°C. The mixtures were inoculated into 96-well microdilution plate cultures of BSC-1 cells and incubated at 37°C for 18 to 20 h. The cultures were fixed and examined by IF. Antibody titers were expressed as the highest dilution neutralizing 50% or more of the IFFU.

(ii) IF. The indirect method of IF has been described previously (27). Goat anti-mouse immunoglobulin G (heavy and light chains) affinity-purified and fluorescein-labeled antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) were used for detection of MAb binding. For identification of the MAb isotype, all the MAbs were tested by IF with goat anti-mouse isotype-specific second antibodies (Kirkegaard and Perry).

(iii) ELISA. The method used for ELISA has been described previously (27), with the modification that goat anti-mouse immunoglobulin G (heavy and light chains) affinity-purified and peroxidase-labeled antibodies (Kirkegaard and Perry) were used. A high titer (>10^8 IFFU/0.1 ml) of infectious virus was prepared and used at a 1:200 or 1:400 dilution as the ELISA antigen.

(iv) IP. Immunoprecipitation (IP) was performed as described by Holland et al. (13), with minor modifications. Briefly, rotavirus-infected cultures without trypsin in the medium were incubated for 5 h at 37°C, actinomycin D was added to 5 μg/ml, and after 1 h, the cells were pulse-labeled with 4 μCi of [^{35}S]methionine per ml for a further hour. The [^{35}S]methionine-labeled soluble antigens were extracted from infected cells by freeze-thawing and RIPA buffer and, after high-molecular-weight complexes had been removed by centrifugation, incubated with MAb or an appropriate antiserum overnight at 4°C. Protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals, Piscataway, N.J.) presensitized with rabbit anti-mouse or anti-bovine immunoglobulins were added and incubated at 4°C for 1 h. The antibody-antigen-protein A complex was centrifuged in a microcentrifuge for 2 min and washed first with RIPA buffer and then sequentially with 1.0 M NaCl (0.01 M Tris hydrochloride, 0.1% Nonidet P-40), with 0.1 M NaCl (1 mM EDTA, 0.01 M Tris hydrochloride, 0.1% Nonidet P-40, 0.3% SDS), and finally with 0.01 M Tris hydrochloride containing 0.1% Nonidet P-40. The pellet was suspended in sample buffer (0.0625 M Tris hydrochloride, 10% glycerol, 2% SDS, 5.0% β-mercaptoethanol, pH 6.8), boiled for 3 min, and pelleted in the microcentrifuge. The viral proteins in the supernatant were analyzed by SDS-PAGE in a 0.75-mm 10% separating gel with a 5% stacking gel run under constant current. The gel was impregnated with scintillant (2,5-diphenyloxazole, PPO), dried under vacuum, and exposed to Kodak XAR-5 film at −70°C.

RESULTS

Electrophoretic characterization of bovine rotavirus genomes and polypeptides. The comparative electrophoretic mobilities of the proteins of the three bovine viruses and the canine virus control are shown in Fig. 1, and their respective RNA electropherotypes are shown in Fig. 2. Distinct differ-

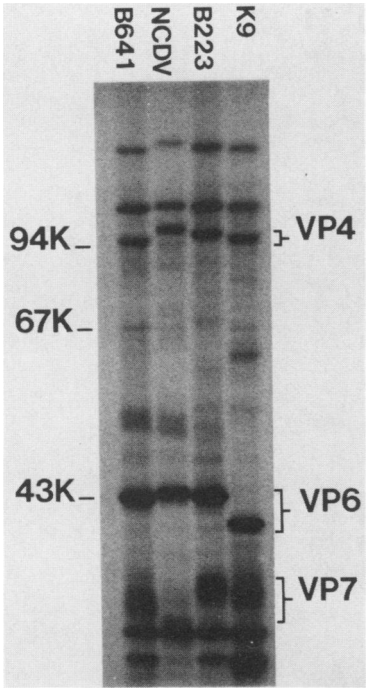

FIG. 1. Comparison of rotavirus-infected cell polypeptide migration patterns by SDS-PAGE. K, 10^3.

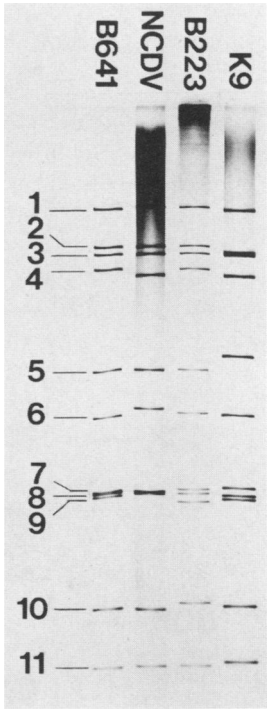

FIG. 2. Comparison of viral RNA electrophoretic migration patterns by SDS-PAGE.

ences were detected in the mobilities of VP4, VP6, and VP7. Differences were also observed in the mobilities of RNA segment 4 (encoding VP4), segment 6 (encoding VP6), and segment 9 (encoding VP7). Electrophoretic polymorphisms were noted for other segments and polypeptide species, but this study concentrated on the segments encoding the polypeptides VP4, VP6, and VP7.

Antigenic relationships among bovine rotavirus polypeptides. The results of comparative NT, IF, ELISA, and IP assays for MAbs generated against B641 and B223 are given in Table 1, and examples of IP are shown in Fig. 3. Neutralizing MAbs are designated with an N suffix, and nonneutralizing MAbs have an E suffix. All the MAbs were of the immunoglobulin G isotype (data not shown).

The neutralizing MAbs were specific by NT for their homologous bovine rotavirus, although one B223 MAb (B223-N6) and one B641 MAb (B641-N1) showed lower-titer cross-reactivity with B641 and canine rotavirus or with canine rotavirus, respectively. The two serotype 6 viruses, B641 and NCDV, apparently do not share all epitopes since MAb B641-N1 only neutralized B641, whereas MAb B641-N2b neutralized both viruses.

The IF test was positive with all the ELISA-reactive (nonneutralizing) MAbs when tested against the three bovine strains and the canine strain. Most of the ELISA-negative (neutralizing) MAbs reacted in IF only with the homologous virus, the exceptional MAbs being B223-N6, which cross-reacted with all four viruses, B641-N1, which cross-reacted with the canine virus, and B641-N2b, which cross-reacted with NCDV. The IF test was found to be the most useful antibody test for early screening of the hybridoma cultures because the hybridomas were positive prior to the first

TABLE 1. Comparative properties of antirotavirus MAbs in NT, IF, ELISA, and IP tests

MAb	NT titer				IF				ELISA titer				IP titer[a]			
	B223	B641	NCDV	K9	B223	B641	NCDV	K9	B223	B641	NCDV	K9	B223	B641	NCDV	K9
B223-E4	<4	<4	<4	<4	+	+	+	+	4,096	8,192	16,384	4,096	6	6	6	6
B223-E8	<4	<4	<4	<4	+	+	+	+	8,192	8,192	8,192	4,096	6	6	6	6
B223-N1	1,024	<4	<4	<4	+	−	−	−	<4	<4	<4	<4	4	−	−	−
B223-N3	2,048[b]	<4	<4	<4	+	−	−	−	<4	<4	<4	<4	4	−	−	−
B223-N4	512[b]	<4	<4	<4	+	−	−	−	<4	<4	<4	<4	4	−	−	−
B223-N6	1,024	4	<4	32	+	+	+	+	<4	<4	8	8	4	4	4	4
B223-N7	6,400	<4	4	<4	+	−	−	−	<4	<4	<4	<4	−	ND[c]	ND	ND
B641-E2	<4	<4	<4	<4	+	+	+	−	512	2,048	4,096	1,024	6	6	6	6
B641-E3	<4	<4	<4	<4	+	+	+	+	<4	64	512	16	7	7	7	7
B641-N1	<4	256	<4	128	−	+	−	+	<4	<4	<4	<4	−	4	−	4
B641-N2b	<4	1,024	1,024	<4	−	+	+	−	<4	<4	<4	<4	−	−	−	−

[a] IP of VP4, VP6, or VP7.
[b] Partial neutralization (70 to 90% IFFU) from 4-1024 (N3) and 4-256 (N4).
[c] ND, Not done.

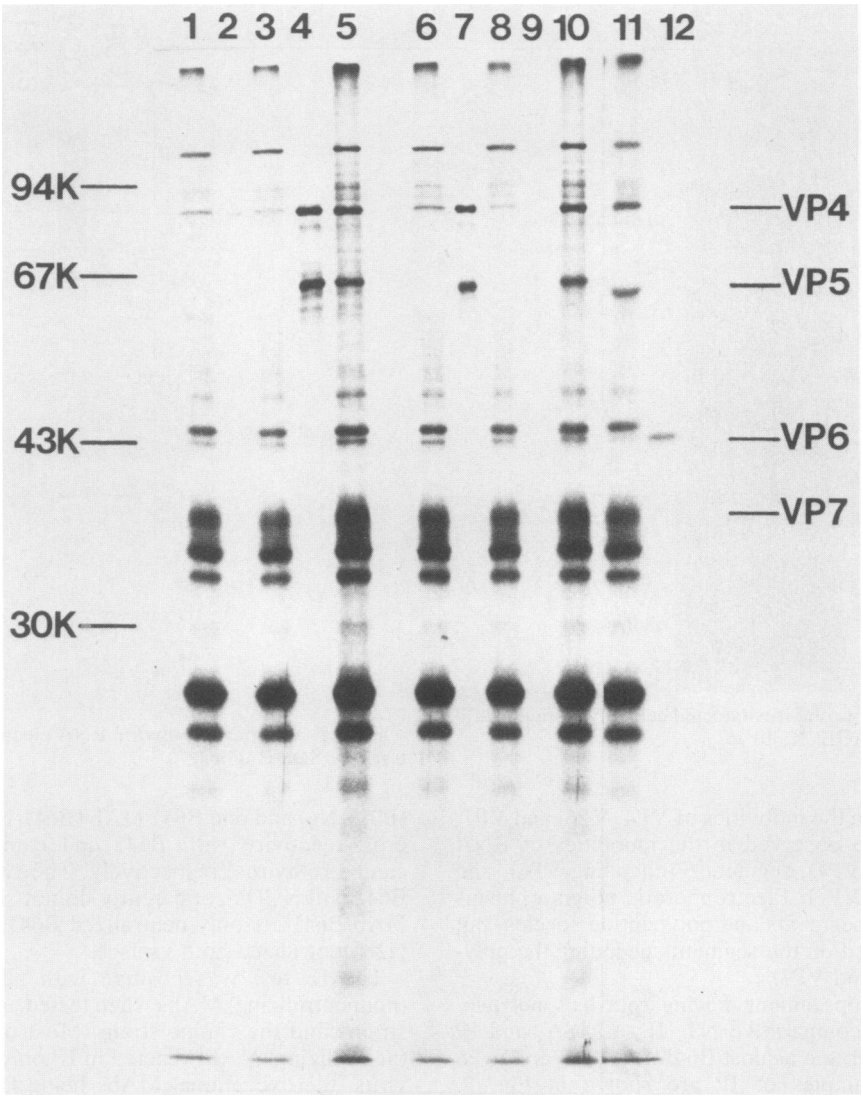

FIG. 3. Example of IP (SDS-PAGE). Controls (not shown) included labeled proteins extracted from uninfected cultures with which the MAbs did not react and IP of viral proteins VP4, VP6, and VP7 with the polyclonal serum GC47. IP of B223-infected cell polypeptides with B223 MAbs: lanes 1 and 2, N1; lanes 3 and 4, N3; lanes 6 and 7, N4; lanes 8 and 9, N6; lanes 11 and 12, E8. Lanes 5 and 10 are control B223-infected cell polypeptides before IP treatment. Of each pair of lanes, the first represents the polypeptides remaining in the supernatant after the IP procedure and the second represents the particular VP immunoprecipitated. For N6 (lane 9), a faint IP band was discernible for VP4 which was confirmed by the reduction of the VP4 band in the supernatant (lane 8). N1, N3, and N4 show IP with VP4 and VP5, and E8 immunoprecipitates with VP6. B223-E8 was run in the same gel in a different region, and for the purpose of this figure, lanes 11 and 12 were moved in the photograph. K, 10^3.

cloning. This permitted the identification of MAbs which only reacted with the homologous rotavirus, a good indication of a neutralizing MAb (Table 1). The ELISA was less sensitive than the NT and IF tests for screening MAbs, except for those reacting with the common rotavirus antigen VP6, and was useful only in screening for nonneutralizing MAbs. With only a single exception, B223-N6, the ELISA was negative for neutralizing MAbs, and that exception was positive only at very low titer. The nonneutralizing MAbs cross-reacted in ELISA, IF, and IP, suggesting little type specificity for ELISA-positive MAbs.

In IP assays, ELISA-positive (nonneutralizing) MAbs precipitated the respective polypeptide species (VP6) across serotypic boundaries, with the exception of B641-E3, which immunoprecipitated VP7 of all four viruses. In contrast, the ELISA-negative (neutralizing) MAbs were generally capable of IP only with the homologous virus. The exceptions were MAb B223-N6, which recognized an epitope on VP4 of all four viruses examined, and MAb B641-N1, which also recognized an epitope on VP4 of the canine virus. The neutralizing MAbs B223-N7 and B641-N2b were particularly interesting because they did not immunoprecipitate with their respective polypeptides. To determine whether B223-N7 was directed against VP4 or VP7, we used it in NTs with reassortant viruses that segregated genome segments 4, 6, and 9 of B223 with SA11-4F (Table 2). MAb B223-N7 neutralized only the reassortant virus that contained segment 9 from the B223 parent and failed to neutralize reassortant clones containing segment 9 from the SA11-4F parent or segment 4 from the B223 parent, indicating that the

TABLE 2. Neutralization of B223/SA11-4F reassortant viruses

Virus	Origin of segment[a]			MAb NT titer	
	4	6	9	B223-N7	B223-N1
B223	B	B	B	3,200	1,024
SA11-4F	S	S	S	<10	<10
5y-159	S	B	B	>1,000	<10
5y-149	B	B	S	<10	1,000
5y-141	S	B	S	<10	<10
6y-76	B	B	S	<10	>100

[a] All segments not shown were derived from the B223 parent, except in the SA11-4F parent, in which all segments were derived from SA11-4F. B and S indicate that the segments were derived from either B223 or SA11-4F virus, respectively.

neutralizing activity of the MAb was directed toward VP7 of B223, the product of segment 9. Reassortants for B641, NCDV, and canine rotaviruses were not available for determining whether B223-N7 or B641-N2b would react with their respective VP7s.

The MAbs B223-N3 and B223-N4 were unusual in that they left a relatively large nonneutralizable fraction of virus, 10% at a 1:4 dilution rising to 50% over 9 to 11 twofold dilutions. The other MAbs behaved like polyclonal sera in that over a range of three to four twofold dilutions, the neutralized fraction fell from 100 to 0%. This observation suggested that the B223 population used for the neutralization tests was heterogeneous for the epitopes recognized by B223-N3 and B223-N4. To investigate this possibility, we incubated approximately 100 IFFU of B223 for 1 h with B223-N1 (1:100 dilution), B223-N3 (1:40), or B223-N4 (1:40). After incubation, cell cultures were inoculated and maintained with the same concentration of MAb in the medium. At each passage, the virus was diluted to 100 IFFU and the relevant MAb treatment was repeated. After passage 4, the resulting viruses (MAb-resistant mutants B223N1, B223N3, and B223N4) were passaged once without MAb treatment and then tested for their susceptibility to neutralization with a panel of MAbs and postinfection serum GC47 (Table 3). Each mutant was no longer neutralizable with the selecting MAb but remained neutralizable with all the other MAbs and with GC47. This result indicated that MAb-resistant mutants had been selected from the B223 population. The electropherotypes of the MAb-resistant mutants were examined to determine whether the selection was accompanied by changes in the genomic RNAs as determined in high-resolution gels. All MAb-resistant mutants had electropherotypes identical to that of the parental B223 virus (data not shown).

DISCUSSION

The results of the electrophoretic analyses of the polypeptides and genome RNAs of the bovine rotaviruses NCDV, B641, and B223 confirmed the results of others (1) which indicate that electrophoretic migration patterns, while useful in epidemiological studies, do not predict serological relationships. Although differences in polypeptide and RNA profiles were noted between serotypes (B223 versus B641 and NCDV), differences of similar magnitude were seen within a serotype (B641 versus NCDV).

The neutralizing MAbs, on the other hand, provided highly specific probes for distinguishing bovine serotypes (B223 versus B641 and NCDV). The neutralizing MAbs confirmed the serotype differences obtained with hyperimmune guinea pig antisera by neutralization (26). It was interesting that one MAb, B223-N6, neutralized to low-titer B641. This MAb may recognize a common neutralizing epitope whose existence may explain the response of B223-vaccinated calves who demonstrate a one-way heterologous serum neutralization of B641 and protection against challenge with B641 (26, 27). In addition, the heterologous neutralization by MAbs B223-N6 and B641-N1 of the canine virus was interesting. Canine rotavirus belongs to serotype 3, and only at high concentrations of hyperimmune guinea pig antisera has cross-neutralization between this serotype and the bovine viruses been obtained (11). However, it has not been determined whether these heterologous reacting antibodies are directed against the same viral antigens (VP4 and VP7) as the homologous reacting antibodies. MAbs generated against serotype 3 viruses did not cross-neutralize a bovine virus of the serotype 6 group (5). Hyperimmunized mice develop high-titer antibody responses which confer passive protection both heterologously and homologously, in contrast to orally infected mice, whose antibody responses are serotype specific (18). It appears that the hyperimmunization response is qualitatively and quantitatively different from the convalescent response and that this may explain the presence of these heterologous reacting MAbs. This conclusion is supported by the detection of an MAb directed against VP4 of porcine OSU rotavirus (serotype 5) which neutralized OSU, RRV (serotype 3), and UK bovine (serotype 6) rotaviruses (19) and of MAbs cross-reactive with VP4 of human rotaviruses of serotypes 1, 3, and 4 (23). Presumably, there are weakly antigenic epitopes on VP4, antibodies which may not be detected in a normal convalescent antibody response. It is possible that VP4 is responsible for the one-way heterotypic neutralization and protection responses seen with some rotaviruses. However, neutralizing MAbs cross-reactive with VP7s of different rotavirus serotypes have also been reported (6, 23). In one of these studies, high-titer ascites fluid MAbs were used, and the differences between homologous and heterologous titers varied from 2- to 300-fold (6). The VP4-reactive MAb B223-N6, which showed weak cross-neutralization with B641 and K9 but cross-reacted by IF and IP with all four viruses, presumably represents common epitopes which are only prominent in B223. The nonneutralizing and VP7-reactive MAb B641-E3 reacted with all four rotaviruses by IF and IP but not by ELISA. Evidence for shared epitopes on VP7 of B223, B641, and NCDV had already been obtained by Rosen (M.S. thesis, Iowa State University, Ames, 1986) using Western blotting (immunoblotting). As this epitope is also on canine rotavirus, it is presumably encoded in a conserved region of genome segment 9 and therefore has no importance in in vitro neutralization reactions.

MAb B641-N2b, which was specific for the serotype 6 bovine rotaviruses B641 and NCDV, did not immunoprecipitate any of the viral proteins. In contrast, MAb B641-N1, reactive with VP4, distinguished between the two viruses.

TABLE 3. NT titers of B223 and B223 MAb-resistant mutants

Virus	NT titer of indicated MAb or serum				
	B223-N1	B223-N3	B223-N4	B223-N7	GC47
B223	2,048	2,048 (4–1,024)[a]	512 (4–256)[a]	6,400	5,120
B223N1	<4	2,048 (4–1,024)[a]	512 (4–256)[a]	>4,096	5,120
B223N3	2,048	<4	512 (4–256)[a]	>4,096	5,120
B223N4	2,048	2,048 (4–1,024)[a]	<4	>4,096	5,120

[a] A 10 to 30% nonneutralizable fraction was obtained over the range of dilutions indicated.

Earlier data had shown that at least one B641-hyperimmunized guinea pig antiserum had a 16-fold difference in NT titer for the two viruses (26). Our data confirm this observation and suggest that either (i) B641 and NCDV do not share a common gene 4 encoding VP4 or (ii) B641 and NCDV express different subsets of epitopes within the serotype 6 epitope set and thus represent different "monotypes" as defined by Coulson (4). The cross-reaction of these two viruses with most hyperimmune antisera and with polyclonal calf antisera suggest that they share most epitopes and differ only at this one epitope or at a small subset of epitopes. It is possible that genome segment 4 and VP4 are responsible for the lack of cross-protection between NCDV and B641 (26), if the epitopes involved in protection were the same as the two serotype 6 monotypes. On the other hand, completely different antigenic forms of VP4 could also be responsible for the absence of cross-protection between B641 and NCDV. To determine the basis for the antigenic relationship between B641 and NCDV, reassortants among them and SA11 are being prepared to identify shared genes and to determine with which gene product, VP4 or VP7, MAb B641-N2b is reactive.

The IF assay proved to be particularly useful for screening MAbs, since it could be performed prior to the first cloning. In addition, by determining the antigenic specificity of the reaction, a prediction could be made whether a particular MAb had neutralizing potential. In this study, the ELISA was less useful than IF, NT, or IP for screening MAbs not reactive with VP6.

MAbs B223-N3 and B223-N4 resulted in a large nonneutralized fraction over a wide range of antibody dilutions, leading us to speculate that the nonneutralized fraction represented monotypic variants (4) within the parental B223 virus population. We were successful in selecting or enriching for these variants and were also able to isolate MAb-resistant mutants to B223-N1, which showed a normal neutralizing pattern. The rapid enrichment for the variants that escaped neutralization with B223-N3 and B223-N4 suggested that these variants existed as a substantial portion of the parental B223 population and are probably common in nature. The homogeneity of the electropherotype of the parental B223 population (26) and the absence of electropherotype variation among these monotype variants serve to emphasize that serological (monotypic) variation can occur in the absence of other, more easily detectable changes and that their demonstration requires the exquisite sensitivity of MAbs. Antigenic variants or mutants similar to B223-N1 have been reported for human rotavirus VP3 (4, 25) and for VP7 (6, 7, 24). However, we have not found a report of MAbs behaving like B223-N3 and B223-N4. Whether monotype variation is important in nature may depend on the particular epitope concerned. The variations reported here probably are not important as they were specific to their particular MAb and they did not escape neutralization by postinfection serum. Another study has shown that similar mutants escaped neutralization by hyperimmune sera but not by postinfection sera (23).

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